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Effect of gamma rays on M_1 attributes and SDS-PAGE analysis in okra [*Abelmoschus esculentus* (L). Moench]

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Abstract

The present study attempts to evaluate the mutagenic effect of gamma rays on okra cultivars of Arka anamika and Arka abhay. The seeds of two traditional cultivars of okra Arka anamika and Arka abhay were treated with different doses of gamma rays like 100, 200, 300, 400, 500 and 600Gy. The gamma irradiation influenced the growth, yield parameters, protein content and banding pattern in M_1 generation. The M_1 growth parameters like germination percentage, survival percentage, plant height and number of leaves per plant and yield parameters such as number of fruits per plant, fruits length, fruit weight, number of seeds per fruit and 100 seeds weight were measured. When compared to control, all the growth and yield parameters were decreased with increasing doses of gamma rays in M_1 generation. The SDS-PAGE analysis of M_1 generation leaf samples of treated population and control showed the appearance and disappearance of some protein bands and the Arka anamika was responded well than Arka abhay in all the terms studied between the two respectively.

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Introduction

Okra (*Abelmoschus esculentus* (L.) Moench] one of the most important vegetables plant everywhere in tropical and warm temperature regions of the world (Patil *et al*., 2015). Okra is a self-pollinating crop belonging to Malvaceae (Oppong - Sekyere *et al*., 2011). The fruits of okra are rich in dietary fiber, minerals (Sodium, Calcium, Sodium, Potassium, Zinc, and Iron), vitamins (A, B, and C), antioxidants, and foliate. They are rich in proteins, the seed oil also edible one enriched with unsaturated fatty acids. Okra mucilage can be used as food additives (NRCNAs 2006; Benchasri 2012; Kumar *et al*., 201; Kumar *et al*., 2017; Gemede *et al*., 2015; Dubey and Mishra 2017).

Mutation breeding is one of the traditional plant breeding methods. The radiations are the best tools to induce genetic variability within a very short span of time. It is highly effective in enhancing natural genetic resources and has been used in developing improved varieties of cereals, fruits and other crops (Lee *et al*., 2002).

Inducing variation through mutation is general and has been found as successful in okra. The special use of induced mutations is basically to improve polygenic traits in crop plants by introducing desirable mutants directly into commercial cultivar, or use them indirectly through cross breeding (Jadhav *et al*., 2013). The uses of mutation breeding to develop the genetic variability for the desired characters in various crop plants and have been proved beyond doubt by a number of scientists (Tah 2006; Adamu and Aliyu 2007; Khan and Goyal 2009; Kozgar *et al*., 2011; Mostafa 2011).

Gamma rays treated with plants are known to create in them hormonal changes, enzymatic alteration, and cell cycle modification in both directions which in turn have effect on morphological characters of plants (Melki and Marouani 2010).

Sodium dodecylesulphate (SDS-PAGE) is widely applied not only for the determination of protein molecular weights and also for the various unknown proteins in addition to the identification and the quantification of newly biosynthesized proteins. Characterization of plant leaf or seed storage proteins by electrophoresis has proved to more rapid and a reliable alternative method for both initial identification and later verification of genotypes (Mohammed 2000).

The aim of this research is to observe the effect of gamma radiation on bhindi [*Abelmoschus esculentus* (L.) Moench] in various growth and yield parameters of M_1 generation with special emphasis on SDS-PAGE analysis. The widespread occurrence of yellow vein mosaic disease in bhindi is a serious issue and affects the successful cultivation. Mutation breeding may be applied to induce disease resistance in this important vegetable crop.

Radiations are the best tool to induce genetic variability within a very short span of time. Induced mutation is a highly effective in enhancing and it has been used in the development of improved cultivars. Physical mutagens are an alternative breeding method to classical breeding methods and genetically modified organisms because of the feet that their applications are relatively safe and cost effective. This method plays a vital role in mutation breeding and could be used for further research process.

Materials and methods

Seed material

The two important traditional varieties of okra (*Arka anamika* and *Arka abhay*) were obtained from Indian Institute of Horticulture Research, Hessaraghatta lake post, IIHR, Main road, Indian Institute of Horticulture Research, Ivarkandapura, Bengaluru, Karnataka 560 089, India.

Gamma Irradiation

The seeds were irradiated with six different dose levels such as 100Gy, 200Gy, 300Gy, 400Gy, 500Gy, and 600Gy and these doses were delivered from Caesium-137 source cell installed at a National Research Centre for Banana, QHJJ+QC2, Somarasempettai-Thogaimalai Road, Thayanur, Trichy, Tamil Nadu 620102, India.

The gamma source was stable and its irradiations were done at a dose rate of 3200 Rads/min by a movable down a cylindrical gasket carrying the seeds.

The experiment was laid out in randomized block design (RBD), with six treatments and control with three replications. There were 150 seeds of each of the six treatments were sown in the field immediately after irradiation at the rate of 20 progeny rows/treatment with proper randomization. Data on qualitative and quantitative characters of M_1 generation were gathered from 25 plants/treatment. The experimental data of M_1 generation was calculated by using OPSTAT software. The mean values of each treatment in three replications were calculated in mean± standard error.

Protein estimation

The estimation of protein was carried out by using Lowry *et al*., (1951) method.

SDS-PAGE analysis

The SDS-PAGE analysis was followed by Laemmli (1970) method.

Results and discussion

Growth and yield parameters

The germination percentage was recorded at 97.52 per cent in Arka anamika and 94.65 in Arka abhay in control seeds. After exposure to gamma rays in both varieties of okra showed reduction in germination. It was 91.21, 87.52, 72.25, 67.37, 50.59 and 41.60 per cent respectively in 100, 200, 300, 400, 500 and 600Gy of gamma rays in Arka anamika followed by 90.10, 79.40, 70.05, 64.39, 48.47 and 40.20 per cent in Arka abhay. On 30th day after showing the survival percentage was calculated in both the varieties of control and treated plants and it was 96.00, 90.23, 80.54, 70.15, 65.33, 46.27 and 40.32 per cent respectively in control, 100, 200, 300, 400, 500 and 600Gy of gamma rays in Arka anamika followed by 93.13, 80.03, 78.51, 68.45, 60.23, 44.63 and 38.79 per cent in Arka abhay**.** On 40th day after showing, the height of the plants was calculated in both the control and gamma irradiation treatments.

There was a gradual reduction in plant height with increasing doses of gamma irradiation. It was 34.51, 32.76, 30.63, 28.35, 27.90, 25.94 and 24.39cm in Arka anamika respectively in control, 100, 200, 300, 400, 500 and 600Gy followed by 32.81, 30.15, 29.26, 26.70, 25.83, 24.80 and 23.70cm in Arka abhay.

The mean number of leaves also decreased with increasing gamma irradiation. In control the total numbers of leaves were 23.31 in Arka anamika 20.24 in Arka abhay. The reductions in number of leaves were noticed in all the treatments in both the varieties.

The number of fruits was 8.83 in Arka anamika and respectively 7.98 in Arka abhay. The numbers of fruits were steadily decreasing with increasing doses of gamma irradiation in both varieties than the control.

The maximum fruit length was observed in control of both varieties. There was a progressive decline in fruit length as a result of exposure to gamma rays in Arka anamika. The length of the fruit ranged from 23.86, 22.08, 21.89, 21.81, 19.03, 18.67 and 17.19 and in Arka abhay it was 20.97, 19.51, 18.59, 18.65, 17.63, 17.46 and 16.29 in control, 100, 200, 300, 400, 500 and 600Gy. In irradiated populations the fruit length showed decreasing trend in both the varieties**.**

The weight of the fruit ranged from 39.81, 32.90, 31.44, 30.37, 30.56, 29.04 to 28.61in Arka anamika and 36.67, 31.96, 30.42, 29.87, 29.60, 26.15 and 24.66 in Arka abhay. In control the numbers of seeds were 72.98 and 69.83 respectively in Arka anamika and Arka abhay.

The weight of the 100 seeds also showed decreasing trend in both the varieties of okra. In Arka anamika 100 seeds weight was 8.40gm, whereas 8.27gm in control of Arka abhay.

In both varieties, the seeds were derived from gamma irradiation showed a decreasing trend with increasing doses of gamma rays. The minimum weights of the 100 seeds were 4.48gm Arka anamika and 4.16gm Arka abhay (Table 1 & 2).

Table 1. Effect of gamma rays on growth characteristics of two traditional cultivars of okra [*Abelmoschus esculentus* (L.) Moench] in M_1 generation.

Dose	Germination percentage		Mean	Survival Percentage		Mean	Plant height		Mean	Number of leaves		Mean
	Arkaanamika	Arkaabhay		Arkaanamika	Arkaabhay		Arkaanamika	Arkaabhay		Arkaanamika	Arkaabhay	
Control	97.52	94.65	96.08	96.00	93.13	94.56	34.51	32.81	33.66	23.31	20.24	21.77
100Gy	91.21	90.10	90.65	90.23	80.03	85.13	32.76	30.15	31.45	21.62	20.65	21.13
200Gy	87.52	79.40	83.46	80.54	78.51	79.52	30.63	29.26	29.94	19.16	17.16	18.16
300Gy	72.25	70.05	71.15	70.15	68.45	69.30	28.35	26.70	27.52	16.13	15.76	15.94
400Gy	67.37	64.39	65.88	65.33	60.23	62.78	27.90	25.83	26.86	14.54	13.49	14.01
500Gy	50.59	48.47	49.53	46.27	44.63	45.45	25.94	24.80	25.37	12.87	10.57	11.72
600Gy	41.60	40.20	40.90	40.32	38.79	39.55	24.39	23.70	24.04	10.96	9.67	10.31
C.D.	-	-	4.18	-	-	5.60	-	-	1.10	-	-	1.62
SE(m)	-	-	1.18	-	-	1.58	-	-	0.31	-	-	0.46
SE(d)	-	-	1.67	-	-	2.24	-	-	0.44	-	-	0.65
C.V.	-	-	2.35	-	-	3.30	-	-	1.55	-	-	4.04

Table 2. Effect of gamma rays on yield characteristics of two traditional cultivars of okra [*Abelmoschus esculentus* (L.) Moench] in M_1 generation.

Dose	Number of fruits per plant		Mean	Fruit Length		Mean	Fruit Weight		Mean	Number of seeds/fruit		Mean	Weight of the fruit / plant		Mean
	Arkaanamika	Arkaabhay		Arkaanamika	Arkaabhay		Arkaanamika	Arkaabhay		Arkaanamika	Arkaabhay		Arkaanamika	Arkaabhay	
Control	8.83	7.98	8.40	23.86	20.97	22.41	39.81	36.67	38.24	72.98	69.83	71.40	8.40	8.27	8.33
100Gy	8.56	7.67	8.11	22.08	19.51	20.75	32.90	31.96	32.43	69.76	62.76	66.20	8.00	7.85	7.92
200Gy	7.99	6.89	7.44	21.89	18.59	20.24	31.44	30.42	30.93	58.36	53.36	55.86	7.10	6.55	6.82
300Gy	7.60	6.70	7.15	21.81	18.65	20.23	30.37	29.87	30.12	56.86	51.45	54.15	6.00	5.75	5.87
400Gy	6.98	5.94	6.46	19.03	17.63	18.33	30.56	29.60	30.05	49.78	49.26	49.52	5.92	5.27	5.59
500Gy	6.56	5.73	6.14	18.67	17.46	18.06	29.04	26.15	27.59	48.70	48.56	48.63	5.81	5.00	5.40
600Gy	6.16	5.66	5.91	17.19	16.29	16.74	28.61	24.66	26.63	47.26	47.20	47.23	4.48	4.16	4.32
C.D.	-	-	0.33	-	-	1.76	-	-	2.42	-	-	5.03	-	-	0.65
SE(m)	-	-	0.09	-	-	0.50	-	-	0.68	-	-	1.42	-	-	0.18
SE(d)	-	-	0.13	-	-	0.70	-	-	0.97	-	-	2.01	-	-	0.26
C.V.	-	-	1.91	-	-	3.62	-	-	3.15	-	-	3.59	-	-	4.13

The results of the study confirmed that the reduction in growth and yield parameters of okra with increased dosage of gamma rays (Jagajanantham *et al.*, 2012). As per the gamma ray treatment, the gradual decrease in growth and yield of okra with increased dosage of gamma rays were reported by several workers in various plants including okra *viz.*, in (Blackgram, Ramaswamy, 1973; soybean, Balakrishnan, 1991; soybean, Pavadai *et al.*, 2010; cowpea, Gnananamurthy *et al.*, 2012; okra, Manivel *et al.*, 2022).

Protein estimation

The leaf samples were collected from M_1 generation of Arka anamika plants from the field. The doses administered were 100 to 600Gy. All the six gamma radiation treatment along with control was subjected to protein estimation on 40th day. In control the amount of protein was 32.18mg/g dry weight, but there was a slight increase in protein content in 100 and 200Gy gamma irradiation (41.26 and 43.25) over control. After that there was a decreasing tendency of protein content up to 600Gy, such as 24.67, 23.25, 22.82 and 21.85mg/g dry weight respectively in 300, 400, 500 and 600Gy. In general based on above findings the lower doses of gamma rays up to 200Gy showed positive effect but 300 to 600Gy recorded negative effects in terms of protein content. Like that of our present research work (Gnanamurthy *et al.*, 2013) reported decreased and increased starch and protein content in *Zea mays* (L.) following treatment with ethyl methane sulphonate.

SDS-PAGE analysis

The electrophoretic analysis of okra (Arka anamika) leaf protein showed slight differences in number of bands recorded. The calculated banding pattern of gamma irradiation treatment showed the following number of bands. In control, the total numbers of bands recorded were five with 12.5, 15.3, 19.5, 25.4, and 49.2 kDa. In 100Gy and 200Gy the total number of bands observed were seven with 12.5, 15.3, 19.5, 25.4, 42.8, 49.2, and 62.4 kDa and the total bands observed were six with 12.5, 15.3, 19.5, 25.4, 42.8, and 62.4kDa. In 300Gy and 400Gy the total numbers of bands noticed were five with 12.5, 15.3, 19.5, 25.4 and 42.8kDa, and the total number of bands four were recorded 12.5, 15.3, 19.5 and 25.4 kDa.

In both 500Gy and 600Gy the total numbers of bands were four with 12.5, 15.3, 19.5 and 25.4 kDa, whereas

the total bands were four with 12.5, 15.3, 19.5 and 25.4 kDa. The 42.8 kDa band was absent in control but existing in 100, 200, and 300Gy. The 49.2 kDa appeared only control followed by 100Gy only. The 62.4 kDa present in 100 and 200Gy but absent in control as well as in 300, 400, 500 and 600Gy (Table 3 and Fig. 1).

Table 3. Effect of gamma irradiation on total number of proteins in the okra plant leaf sample cv- Arka anamika in M_1 generation.

kda value	Amount of protein (mg/g dry weight)	Control	100Gy	200Gy	300Gy	400Gy	500Gy	600Gy
12.5	32.18	+	+	+	+	+	+	+
15.3	41.26	+	+	+	+	+	+	+
19.5	43.25	+	+	+	+	+	+	+
25.4	24.67	+	+	+	+	+	+	+
42.8	23.25	-	+	+	+	-	-	-
49.2	22.82	+	+	-	-	-	-	-
62.4	21.85	-	+	+	-	-	-	-

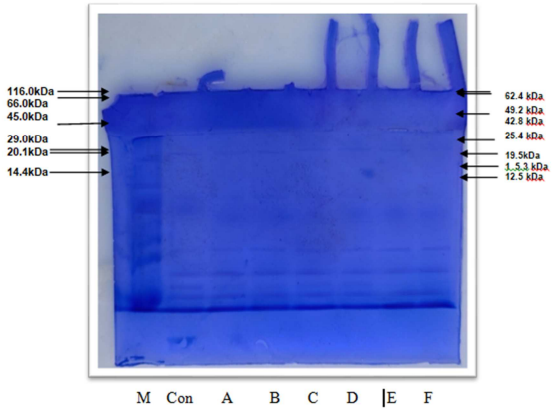

Fig. 1. Effect of gamma rays on SDS-PAGE analysis of cv- Arka anamika in M_1 generation.
(M-Marker ; Con- Control; A-100Gy; B-200Gy; C-300Gy; D-400Gy; F-500Gy; E-600Gy)

The same trend was supported by (Rashed *et al.*, 1994) they found that gamma rays cause modulation in protein patterns by inducing appearance and/or disappearance of some protein bands. In okra gamma irradiation induced protein polymorphism was reported by (Hegazi and Hamideldin 2010). They observed the number of polymorphic bands among the two varieties under investigation is fifteen. In Sabahia, variuos treatments induced changes in protein pattern.

Conclusion

The present investigations revealed that the effect of gamma rays on growth, yield and different protein banding pattern of cultivars of okra. Among the different treatments all the M_1 populations showed decreasing tendency along with increasing doses/concentrations of gamma rays. Based on the investigation growth and yield were most effective in Arka anamika than Arka abhay. The SDS-PAGE analysis showed the influence of gamma rays on variations in banding pattern. Hence all these treatments could be used in plant breeding programs for inducing viable mutants in okra.

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Conflict of interest

The authors declare that there is no conflict of interests.

References

Adamu AK, Aliyu H. 2007. Morphological effects of sodium azide on tomato (*Lycopersicon esculentum* Mill.). Science World Journal **2(4),** 9-12.

Balakrishnan PC. 1991. Induced mutagenesis in soybean (*Glycine max* (L.) Merrill) Ph.D. Thesis, Tamil Nadu Agriculture University, Coimbatore.

Benchasri S. 2012. Okra (*Abelmoschus esculentus* (L.) Moench) as a valuable vegetable of the world. Ratarstvoi Povrtarstvo **49,** 105-112.

Dubey P, Mishra S. 2017. A review on: Diabetes and okra [*Abelmoschus esculentus* (L.) Moench]. Journal of Medicinal Plants Studies **5,** 23-26.

Gemede HF, Ratta N, Haki GD, Woldegiorgis AZ, Beyene F. 2015. Nutritional Quality and Health Benefits of Okra (*Abelmoschus esculentus*): A review. Journal of Food Process Technology **6,** 458.

Gnanamurthy S, Dhanavel D, Bharathi T. 2013. Effect of chemical mutagenesis on biochemical activity and variability, heritability and genetic advance in *Zea mays* (L) International Journal of Current Science **5,** 57-61.

Gnanamurthy S, Mariyammal S, Dhanavel D, Bharathi T. 2012. Effect of gamma rays on yield and yield components characters R_3 generation in cowpea (*Vigna unguiculata* (L.). Walp.). International Journal of Research and Plant Science **2(2),** 39-42.

Hegazi AZ, Hamideldin N. 2010. The effect of gamma irradiation on enhancement of growth and seed yield of okra [*Abelmoschus esculentus* (L.) Moench] and associated molecular changes. Journal of Horticulture and Forestry **2(3),** 038-051.

Jadhav PA, Kalpande HV, Abad SK, Mali AR. 2013. Induced mutagenesis in okra [*Abelmoschus esculentus* (L.) Moench] by gamma rays and ethyl methane sulphonate. Vegetable Science **40(2),** 223-224.

Jagajanantham N, Dhanavel D, Pavadai P, Chidambaram AI.A. 2012. Growth and yield parameters using gamma rays in bhindi [*Abelmoschus esculentus* (L.) Moench]. var. arkaanamika. International Journal of research in Plant Science **2(4),** 56-58.

Khan S, Goyal S. 2009. Improvement of mung bean varieties through induced mutations. African Journal of Plant Science **3,** 174-180.

Kozgar MI, Goyal S, Khan S. 2011. EMS induced mutational variability in *Vigna radiate* (L) and *Vigna mungo* (L). Research Journal of Botany **6,** 31-37.

Kumar S, Parekh MJ, Fougat RS, Patel SK, Patel CB, Kumar M. 2017. Assessment of genetic diversity among okra genotypes using SSR markers. Journal of Plant Biochemistry and Biotechnology **26,** 172-178.

Laemmli UK. 1970. Cleavage of structural protein during the assembly of the head of bacteriophage T4. Nature **227,** 680-685.

Lee YI, Lee IS, Lim YP. 2002. Variation in sweet potato regenerates from gamma-rays irradiated embryogenic callus. Journal of Plant Biotechnology **4(4),** 163-170.

Lowry OH, Rosebrough AL, Farr and Randall RJ. 1951. Protein measurement with the folin phenol reagent. Journal of Biological Chemistry **193,** 265-275.

Manivel P, Prabakaran V, Parvathi S, Palanivel S. 2022. Effect of gamma rays on growth and GC-MS analysis in bhindi [*Abelmoschus esculentus* (L.) Moench]. Indian Journal of Natural Sciences **13(74),** 49195-49201.

Melki M, Marouani A. 2010. Effects of gamma rays irradiation on seed germination and growth of hard wheat. Environmental Chemistry Letters **8,** 307-310.

Mohamed IFF. 2000. Characterization of some local varieties of Cucurbitaceae growing under Egytian conditions. M. Sc. Thesis, Faculty of Agriculture, Ain-Shams University.

Mostafa GG. 2011. Effect of sodium azide on the growth and variability induction in (*Helianthus annuus* L.) International Journal of Plant Breeding and Genetics **5(1),** 76-85.

NRCNAs. 2006. "Okra". Lost Crops of Africa: vol II: Vegetables. Lost Crops of Africa.2.Washington, D.C: National Academies Press.

Oppong-Sekyere D, Akromah R, Nyamah EY, Brenya E, Yeboah S. 2011. Characterization of okra (*Abelmoschus* spp. L.) germplasm based on morphological characters in Ghana. Journal of Plant Breeding and Crop Science **3(13),** 367-378.

Patil P, Sutar S, Joseph JK, Malik S, Rao S, Yadav S. 2015. A systematic review of the genus Abelmoschus (Malvaceae). Rheedea **25(1),** 14-30.

Pavadai P, Girija M, Dhanavel D. 2010. Effect of gamma rays on some yield parameters and protein content of soybean in M_2, M_3 and M_4 generation. Journal of Experimental Science **1(6),** 8-11.

Ramasamymm. 1973. Investigation on induced mutagenesis in Blackgram Ph.D. Thesis, Tamil Nadu Agriculture University. Coimbatore.

Rashed MA, Fahmy, EM, Sallam MA. 1994. Embryo culture, protein and isoenzyme electrophoresis as selectable marker to predict salt tolerance in wheat. 5th Conf. Agricultural Development Research Faculty of agriculture, Ain Shams Uni. Cario, Egypt **1,** 469-490.

Sathish Kumar D, Eswar Tony D, Praveen Kumar A, Ashok Kumar K, Bramha Srinivasa Rao D, Ramarao N. 2013. A review on: *Abelmoschus esculentus* (Okra). International Research Journal of Pharmaceutical and Applied Sciences **3(4),** 129-132.

Tah PR. 2006. Induced macro mutation in mungbean (*Vigna radiate* (L.) Wilczek). International Journal of Botany **2,** 219-228.